DETERMINATION OF THE DISTRIBUTION OF PYRUVATE CARBOXYLASE IN RAT LIVER

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1. Introduction

We report the presence of sufficient extramitochondrial (soluble) pyruvate carboxylase (PC) in rat liver to account for the observed rate of gluconeogenesis. It has been previously accepted that this enzyme is located in the mitochondria together with glutamate dehydrogenase (GDH). We have used GDH as a mitochondrial marker enzyme for studying the cellular distribution of PC in rat liver, and present evidence in this paper for the existence of significant quantities of extramitochondrial pyruvate carboxylase. Walter et al. [1] and Bottger et al. [2] have previously claimed that the bulk of PC activity is located within the mitochondria. Our results are in direct contradiction to this view. Not only we but other authors have also found appreciable amounts of PC in the soluble extramitochondrial fraction [3, 4]. Walter and Anabitarte however have sought to explain our results by assuming that i) GDH is released less well from the mitochondria than PC during homogenization of the liver. ii) During homogenization equal amounts of GDH and PC are liberated in the cytosol where the GDH is unstable due to the presence of sodium acetate (medium) whereas PC retains its activity. We have excluded these possibilities by showing that (1) breakage of mitochondria by different methods always leads to a simultaneous release of PC and GDH. (2) We have not employed sodium acetate medium in our extraction methods.

This result and the different behaviour of both enzymes with sucrose medium containing sodium ions show clearly that PC is not located exclusively in the mitochondria containing GDH, supporting the concept of an extramitochondrial localization of PC.

2. Methods and material

The homogenization and preparation of the cytosols from livers were carried out according to Henning et al. [5]. The liver was homogenised in isotonic solution containing 0.1 M Tris-HCl buffer pH 7.2; 0.25 M sucrose (2+8, v/v); 1 mM EDTA and 1 mM glutathione. The homogenized liver was centrifuged until clear and the activity was measured in the

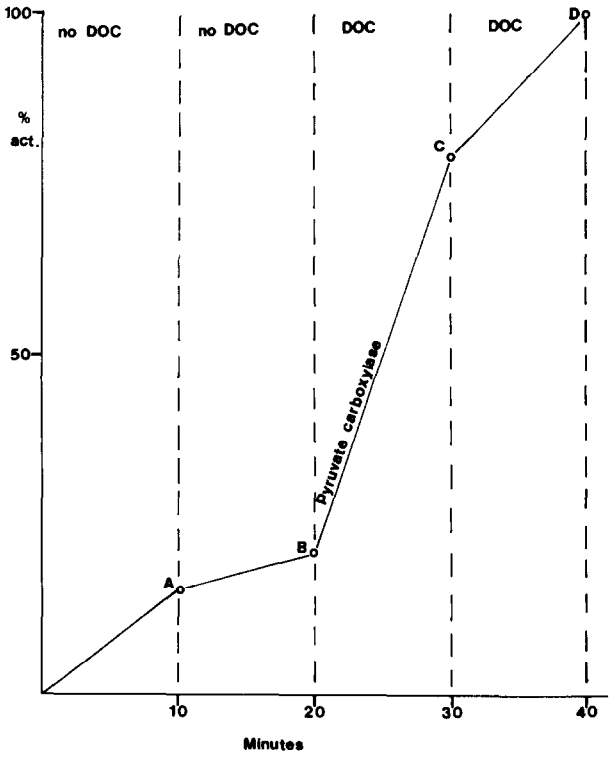

Fig. 1. Extraction of pyruvate carboxylase from rat liver.

supernatant. The total activities of these enzymes were also measured in sonicated homogenate or in desoxycholic acid (DOC-0.1% final conc.) treated liver homogenate. These activities were considered as 100%. This small quantity of DOC did not affect the enzyme (GDH and PC) activity in any way. PC was measured by the method of Henning et al. [5] by transforming the oxalacetate formed from pyruvate by the soluble PC to citrate, and then estimating radioactive citrate in the Packard counter. GDH was measured according to Schmidt [6] in the presence of 3 μmoles ADP.

Coenzymes and enzymes were purchased from Boehringer (W. Germany). All the other reagents were of the highest purity commercially obtainable. Fresh liver was used for each experiment. All solutions were freshly prepared prior to the experiments.

3. Results and discussion

For these experiments liver was taken from non-fasted rats as well as fasted rats, to avoid the possibility that the PC activity in the soluble fraction is due to mitochondria from fasted rats being more fragile and releasing enzymes in a different pattern (more PC and less GDH) during homogenization.

Fig. 1 shows that the main part of the PC activity is extracted from rat liver after adding 0.1% final concentration of DOC to the solution and this 80% activity suggests damaged mitochondria. To investigate on the soluble PC and the mitochondrial damage, GDH was selected as the mitochondrial marker enzyme and as a criterion for the damage of the mitochondria, not only in rat liver but also in porcine and bovine liver etc. Points A and B in fig. 1 show the soluble part of PC after two extractions of the rat liver with our medium. Points C, D show the remaining activity after optimal extraction with the same medium containing 0.1% DOC. As fig. 1 clearly shows, after two extractions of liver with salt containing isotonic (sucrose) medium not less than 20% of the total activity of soluble PC was extracted.

Upon extraction in the presence of Na^+ ions (without DOC) the percentage activity of PC is consistently higher in comparison to that of GDH, but

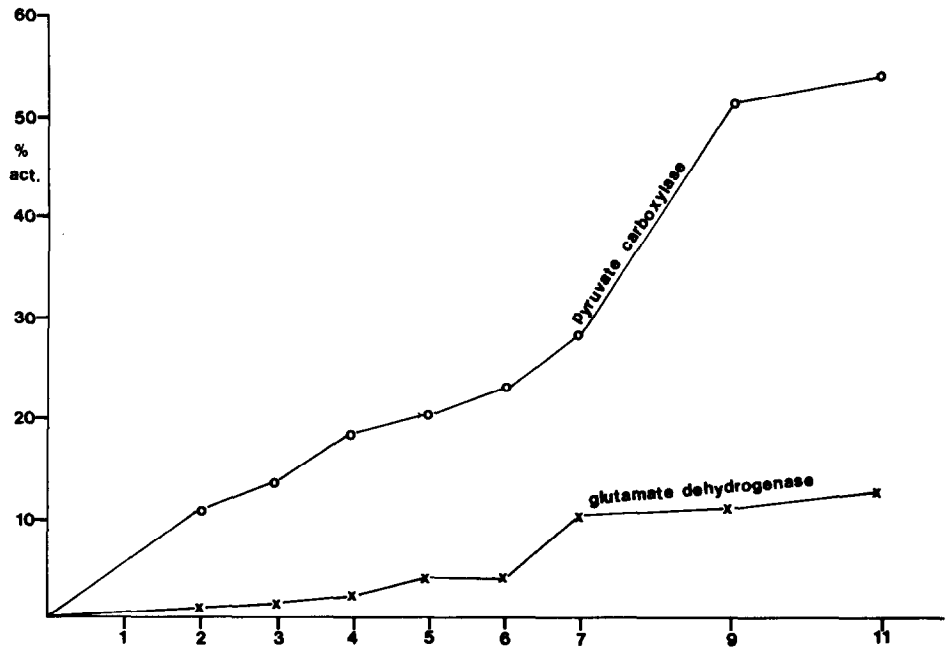

Fig. 2. Extraction of pyruvate carboxylase and glutamate dehydrogenase from rat liver at different times, with isotonic solution containing monovalent cations.

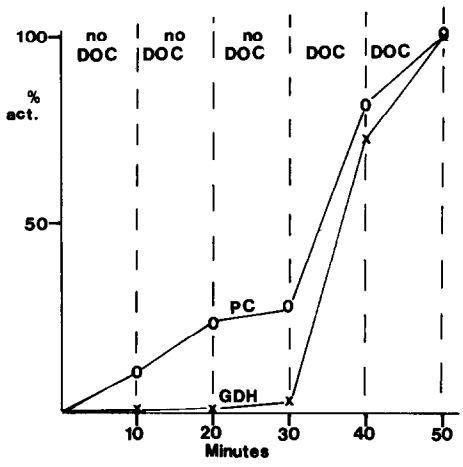

Fig. 3. Fractional extraction of porcine liver with sucrose medium supplemented with monovalent cations.

both have the same percentage activity when extracted in the presence of 0.1% DOC. To investigate the possibility that during this short homogenization GDH remains attached to the tissues, we extracted rat liver and found after 6 min of extraction 23% PC and only 3% of GDH activity in the homogenate and after 11 min 54% soluble PC activity in comparison to GDH with only 11% (fig. 2). This clearly different susceptibility to extraction of both enzymes under the same conditions seems to support our hypothesis of "a different localization of these two enzymes". Evidence was also found for soluble PC in porcine liver (fig. 3) and in chicken liver [7] a good raw material for the isolation of this enzyme.

In our opinion gluconeogenesis from pyruvate does not depend on exclusively intramitochondrial carboxylation of pyruvate, contrary to the original statement of mitochondrial location of this enzyme. We have reached this conclusion on the basis of the different solubilities of GDH and PC upon extraction of rat liver with isotonic solutions supplemented by monovalent cations.

Extraction of liver and also liver mitochondria (which are a mixture of all kinds of subcellular units like microbodies, lysosomes, mitochondria and other unknown compartments) with our medium results in solubilization of a large part of PC. GDH being exclusively intramitochondrial, is extracted only to a small degree. A soluble form of PC has also been described by others [3, 8, 9]. At this point we would like to stress that we prefer the term extramitochondrial location and not cytosolic. The activity of the soluble PC is obtained optimally by using salt containing isotonic solutions. This corresponds to about 30–50% of the total activity of this enzyme in the liver. GDH, an enzyme exclusively located in mitochondria, is only solubilized by this treatment to about 3–10% of the total activity present in the liver. This different behaviour of PC and GDH extracted from liver could be the result of: i) different solubilities of both enzymes of mitochondrial origin; ii) a possible location of PC in an extramitochondrial compartment as well as in the mitochondria.

Different solubilities of mitochondrial PC and GDH could be excluded. As is evident from fig. 4, extraction of mitochondria (with buffered isotonic solution) through Waring blendor results in solubilization of

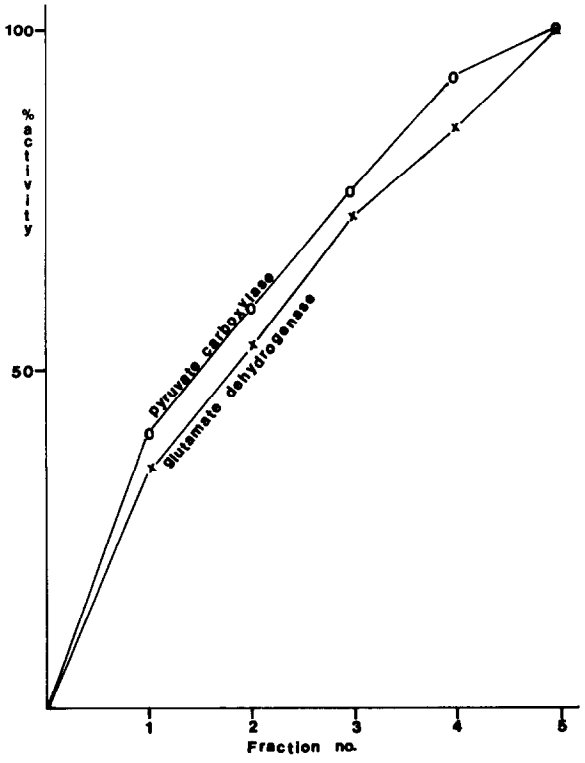

Fig. 4. Extraction through Waring blendor, where the mitochondria and the other compartment containing the soluble PC are damaged. The sum of these different fractions (homogenized at different times) is considered as 100%.

both enzyme activities to the same degree. An extramitochondrial location of PC is evident from the different behaviour of the latter enzyme and GDH on extraction with isotonic solution supplemented by various additions.

However, it seems from the present results that carboxylation of pyruvate is not exclusively restricted to the mitochondrial compartment of rat liver (fig. 5) as postulated by others [10–12]. A possible location of PC in the cytoplasm and in the cristae of the mitochondria was refuted by Wieland and co-workers [2, 13]. Therefore the existence of a second mitochondrial population, or a new compartment containing the soluble PC, seems likely. If the soluble PC were localized in the matrix containing GDH and if we have been damaging these mitochondria during our experiments, then both of the enzymes must appear in the solution (fig. 4) and not as shown in figs. 1 and 2, where the breakage of mitochondria takes place on DOC extraction. Since the solubility of the PC is favoured in presence of monovalent cations while GDH activity remains unchanged, it is possible either that the soluble PC is localised in some new compartment which does not contain GDH or that there are different mitochondrial populations of which only one sort is fragile and that we are able to open this mitochondrion with our method and medium. The negligible quantity of GDH comes from the probable damage of the second type of mitochondrion containing both GDH and PC. Furthermore we obtain the soluble PC after drastic homogenisation, which indicates that the enzyme is not attached loosely to the membrane, otherwise it would be completely released. On the other hand the appearance of GDH is a widely accepted criterion (fig. 4) for the mitochondrial damage. It is difficult to reconcile the different solubilities of PC and GDH with the model in which PC, like GDH, is located only in the matrix. Figs. 1–3 and fig. 4 provide a clear contrast in the mitochondria containing GDH macerated with a Waring blendor or DOC and in the other case the damage is negligible. After breaking the mitochondria, we found the same solubility of PC and GDH. If one assumes that during homogenization, equal amounts of GDH and PC are released into the cytosol where the GDH is unstable, whereas PC retains its activity, under these circumstances it becomes more difficult to explain their different behaviour in sucrose medium. Moreover for the enzyme extraction (mentioned here) we have never used sodium acetate medium.

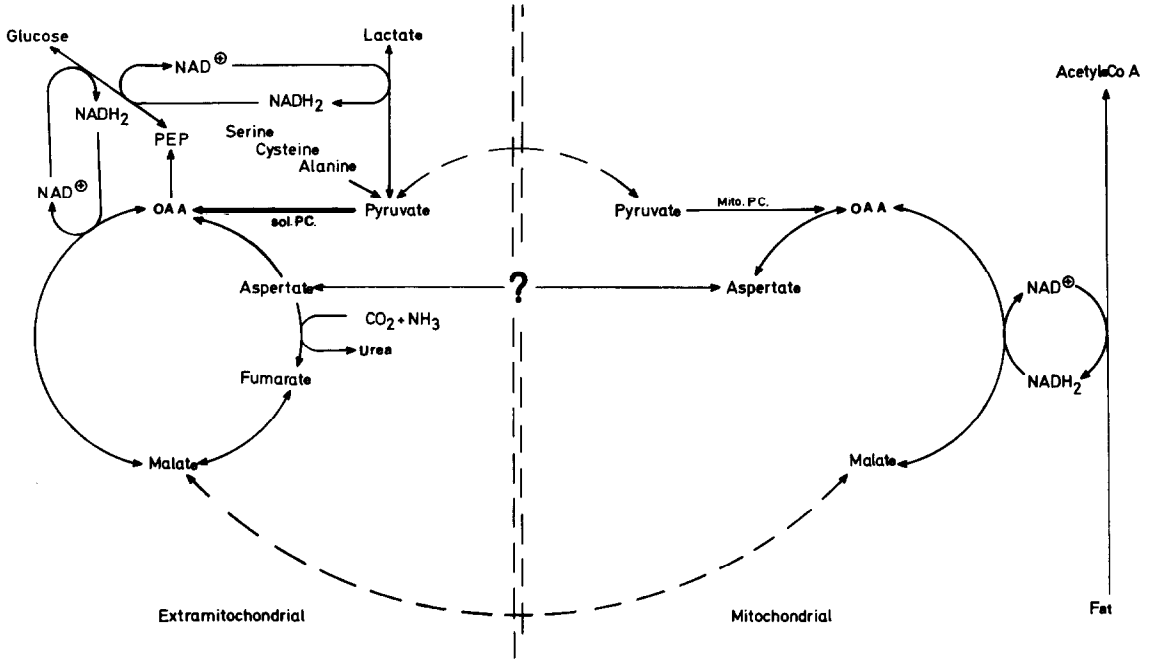

Fig. 5. Hydrogen transport and the extramitochondrial carboxylation of pyruvate through the soluble pyruvate carboxylase.

The mechanism postulated by various authors for the intracellular regeneration and transport of hydrogen are summarised in fig. 5. This scheme differs from those published in one respect, namely an additional possible carboxylation of pyruvate in the extramitochondrial compartment. This assumption seems justified on the basis of our studies. Extramitochondrial carboxylation of pyruvate also seems meaningful in view of NAD^+ regeneration systems located extramitochondrially which could supply the hydrogen necessary for gluconeogenesis from C_3 units. Our results strongly support the view that PC is not exclusively a mitochondrial enzyme in rat liver.

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